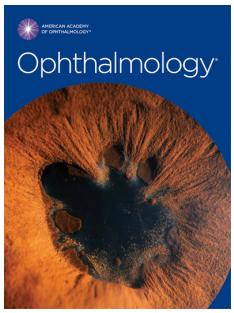
DOI:
10.1016/j.ophtha.2018.09.045

Document Version
Peer reviewed version

Accepted Manuscript



Increased High Density Lipoprotein-levels associated with Age-related Macular degeneration. Evidence from the EYE-RISK and E3 Consortia

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PII: S0161-6420(18)31091-1

DOI: 10.1016/j.ophtha.2018.09.045

Reference: OPHTHA 10513

To appear in: *Ophthalmology*

Received Date: 21 April 2018

Revised Date: 1 September 2018

Accepted Date: 11 September 2018

Please cite this article as: Colijn J, Hollander AId, Demirkan A, Cougnard-Grégoire A, Verzijden T, Kersten E, Meester M, Merle B, Papageorgiou G, Ahmad S, Mulder M, Costa M, Benlian P, Bertelsen G, Bron A, Claes B, Creuzot-Garcher C, Erke M, Fauser S, Foster P, Hammond C, Hense H, Hoyng C, Khawaja A, Korobelnik J, Piermarocchi S, Segato T, Silva R, Souied E, Williams K, van Duijn C, Delcourt C, Klaver C, for theE3 Consortium and EYE-RISK Consortium, Increased High Density Lipoprotein-levels associated with Age-related Macular degeneration. Evidence from the EYE-RISK and E3 Consortia, *Ophthalmology* (2018), doi: https://doi.org/10.1016/j.ophtha.2018.09.045.

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Running head: High HDL associated with AMD

FINANCIAL SUPPORT:

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 634479
The Alienor-3C study received financial support from Laboratoires Théa (Clermont-Ferrand, France), Fondation Voir et Entendre (Paris, France) and Caisse Nationale de Solidarité pour l'Autonomie (Paris, France). Laboratoires Théa participated in the design of the study, but no sponsor participated in the collection, management, statistical analysis and interpretation of the data, nor in the preparation, review or approval of the present manuscript.

The Coimbra Eye Study is an Investigator Initiated Study sponsored by AIBILI that was financially supported by Novartis Pharma AG. The funding organization played no role in the design or conduct of this research.

EPIC-Norfolk infrastructure and core functions are supported by grants from the Medical Research Council (G1000143) and Cancer Research UK (C864/A14136). The clinic for the third health examination was funded by Research into Ageing (262). Genotyping was funded by the Medical Research Council (MC_PC_13048). Mr Khawaja is supported by a Moorfields Eye Charity fellowship. Professor Foster has received additional support from the Richard Desmond Charitable Trust (via Fight for Sight) and we were also supported by the Department for Health through the award made by the National Institute for Health Research to Moorfields Eye Hospital and the UCL Institute of Ophthalmology for a specialist Biomedical Research Centre for Ophthalmology.

EUGENDA was funded by grants from the Oogfonds, MaculaFonds, Landelijke Stiching voor Blinden en Slechtzienden, Stichting Blindenhulp, Stichting A.F. Deutman Oogheelkunde Researchfonds, the Netherlands Organization for Scientific Research (Vidi Innovational Research Award 016.096.309) , and the European Research Council under the European Union's Seventh Framework Programme (FP/2007-2013) (ERC Grant Agreement n. 310644 MACULA).

MONRACHET Funding was provided by an Inter-regional grant (PHRC) and the Regional Council of Burgundy. This study was also funded by INRA, CNRS, Université de Bourgogne, Regional Council of Burgundy France (PARI Agrale 1), FEDER (European Funding for Regional Economic Development) and French Government grant managed by the French National Research Agency (ANR) as part of the "Investissements d'Avenir" program (reference ANR-11-LABX-0021-01-LipSTIC Labex). The funding organizations had no role in the design or conduct of this research.

The POLA study was supported by the Institut National de la Santé et de la Recherche Médicale (Inserm), Paris, France; by grants from the Fondation de France, Department of Epidemiology of Ageing, Paris, the Fondation pour la Recherche Médicale, Paris, the Région Languedoc-Roussillon, Montpellier, France and the Association Retina-France, Toulouse; and by financial support from Rhônes Poulenc, Essilor, Specia and Horiba ABX Montpellier, and the Centre de Recherche et d'Information Nutritionnelle, Paris. The sponsors and funding organizations played no role in the design or conduct of this research.

The Rotterdam Study is funded by Erasmus Medical Center and Erasmus University, Rotterdam, Netherlands Organization for the Health Research and Development (ZonMw), the Research Institute for Diseases in the Elderly (RIDE), the Ministry of Education, Culture and Science, the Ministry for Health, Welfare and Sports, the European Commission (DG XII), and the Municipality of Rotterdam. The authors are grateful to the study participants, the staff from the Rotterdam Study and the participating general practitioners and pharmacists. Additionally, the ophthalmic research within the Rotterdam Study was supported by the following foundations: Oogfonds, Landelijke Stichting voor Blinden en Slechtzienden, Novartis Foundation and MaculaFonds that contributed through UitZicht (grants 2015-36). The funding organizations had no role in the design or conduct of this research and provided unrestricted grants.

Caroline Klaver is consultant for Bayer, Laboratoires Théa, Novartis.
Cécile Delcourt is consultant for Allergan, Bausch+Lomb, Laboratoires Théa, Novartis and Roche.
Rufino Silva is consultant for Alimera, Allergan, Alcon, Bayer, Novartis, THEA.
Alain M. Bron reports personal fees from Allergan, personal fees from Bausch Lomb, grants from Horus, personal fees from Théa, personal fees from Carl Zeiss Meditec, outside the submitted work.
Catherine Creuzot-Garcher reports grants and personal fees from Allergan, personal fees from Bayer, personal fees and other from Novartis, grants from Horus, grants and personal fees from Thea, outside the submitted work.
Anneke den Hollander is a consultant for Ionis Pharmaceuticals.

ABBREVIATIONS

AMD = Age-related macular degeneration; **ABCA1** = ATP-binding cassette (ABC) transporter A1; **(Apo)E =** Apolipoprotein E; **CETP** = Cholesteryl transfer protein; **LIPC** = Lipase C; **E3** = European Eye Epidemiology; **RPE** = retinal pigment epithelium; **VLDL** = very low density lipoproteins; **IDL** = intermediate density lipoproteins; **LDL** = low density lipoproteins; **HDL**= high density lipoproteins; **NMR**= nuclear magnetic resonance; **EPIC** = European Prospective Investigation into Cancer and Nutrition**; ALIENOR** = Antioxydants, Lipids Essentiels, Nutrition et maladies OculaiRes Study; **POLA**= Pathologies Oculaires Liées à l'Age Study**, EUGENDA** = European genetic database; **RS** = Rotterdam Study; **BMI** = body mass index; **PAMDI** = Prevalence of Age-related Macular Degeneration in Italy; **EDTA** = Ethylenediaminetetraacetic acid; **SNP**= single nucleotide polymorphism; **OR** = Odds Ratio; **HR** = Hazard Ratio; **LCAT** = lecithin-cholesterol acyltransferase;

This article contains additional online-only material. The following should appear online-only: Figure 4 , Tables 1, 4-19 and cohort descriptions.

ABSTRACT

Purpose: Genetic and epidemiologic studies have shown that lipid genes and High Density Lipoproteins (HDL) are implicated in age-related macular degeneration (AMD). We studied circulating lipid levels in relation to AMD in a large European dataset, and investigated whether this relationship is driven by certain sub fractions.

Design: (Pooled) analysis of cross-sectional data.

Participants: 30,953 individuals aged 50+ participating in the E3 consortium; and 1530 individuals from the Rotterdam Study with lipid sub fraction data.

Methods: In E3, AMD features were graded per eye on fundus photographs using the Rotterdam Classification. Routine blood lipid measurements were available from each participant. Data on genetics, medication and confounders such as body mass index, were obtained from a common database. In a subgroup of the Rotterdam Study, lipid sub fractions were identified by the Nightingale biomarker platform. Random-intercepts mixed-effects models incorporating confounders and study site as a random-effect were used to estimate the associations.

Main Outcome Measures: early, late or any AMD, phenotypic features of early AMD, lipid measurements.

Results: HDL was associated with an increased risk of AMD, corrected for potential confounders (Odds Ratio (OR) 1.21 per 1mmol/L increase (95% confidence interval[CI] 1.14-1.29); while triglycerides were associated with a decreased risk (OR 0.94 per 1mmol/L increase [95%CI 0.91-0.97]). Both were associated with drusen size, higher HDL raises the odds of larger drusen while higher triglycerides decreases the odds. LDL-cholesterol only reached statistical significance in the association with early AMD (p=0.045). Regarding lipid sub fractions: the concentration of extra-large HDL particles showed the most prominent association with AMD (OR 1.24 [95%CI 1.10-1.40]). The *CETP* risk variant (rs17231506) for AMD was in line with increased-HDL levels ($p=7.7x10^{-7}$); but *LIPC* risk variants (rs2043085, rs2070895) were associated in an opposite way ($p=1.0x10^{-6}$ and $1.6x10^{-4}$).

Conclusions: Our study suggests that HDL-cholesterol is associated with increased risk of AMD and triglycerides negatively associated. Both show the strongest association with early AMD and drusen. Extra-large HDL sub fractions seem to be drivers in the relation with AMD, variants in lipid genes play a more ambiguous role in this association. Whether systemic lipids directly influence AMD or represent lipid metabolism in the retina remains a question to be answered.

Word count: 350

Keywords: Age-related macular degeneration , lipids, high-density lipoproteins, cholesterol, E3 Consortium

INTRODUCTION

Age-related macular degeneration (AMD) is a leading cause of blindness in the developed world with 10.4 million sufferers worldwide in 2015[1]. It is a multifactorial disease affecting the elderly involving genetics and lifestyle factors. The diagnosis of AMD is based on imaging of the retina with drusen as the hallmark of early disease, and chorioretinal neovascularization and atrophy of the retinal pigment epithelium (RPE) are indicative of late disease. The number of drusen and total drusen area are prominent predictors of progression of the early stages of AMD[2, 3].

Drusen are lipid-rich, protein-containing deposits that accumulate between the RPE and Bruch's membrane. The accumulation of drusen shows resemblance to the formation of atherosclerotic plaques[4] seen in cardiovascular disease, with a similar composition of proteins and protein complexes such as apolipoprotein (Apo)E, cholesterol esters and complement proteins.[5, 6] The lipid load in drusen is as high as 40%[7], and is thought to be partly derived from the systemic circulation. This triggered many studies to evaluate the relationship between serum or plasma lipids and AMD.[8-12] Some found associations with various serum or plasma lipid levels and drusen or AMD[11-18], but results were mainly weak and inconsistent. As a biological explanation is lacking, the relationship remains unsettled but intriguing.

Genetically, lipid metabolism is also involved in AMD. Genetic associations have been established for four genes encoding components of the HDL metabolism: *ABCA1*, *CETP*, *APOE* and *LIPC*[19-25]. The ATP-binding cassette (ABC) transporter A1 (ABCA1) is a cellular cholesterol efflux pump leading to formation of nascent HDL. ApoE, encoded by the *APOE* gene, facilitates cholesterol uptake by HDL. Cholesteryl transfer protein (CETP) exchanges cholesteryl esters and triglycerides between HDL and other lipoproteins, and thereby influences HDL particle size.[26] Lastly, hepatic lipase encoded by the *LIPC* gene hydrolyzes triglycerides and phospholipids in lipoproteins[27] and thereby partly converts very low density lipoproteins (VLDL) and intermediate density lipoproteins (IDL) to low density lipoproteins (LDL)[20] and plays a role in altering the HDL contents.

The European Eye Epidemiology (E3) consortium within the European EYE-RISK project enabled us to investigate the relationships between systemic lipids levels, lipid genes, and AMD using a very large data set. With nuclear magnetic resonance (NMR) spectroscopy we studied these relationships in greater detail to investigate which particles are driving potential associations.

METHODS

Study population:

Routine blood lipid measurements

Fourteen studies from France, Germany, Italy, the Netherlands, Norway, Portugal and United Kingdom participating in the E3 consortium enrolled in the current study (cohort descriptions available at External link http://www.aaojournal.org). E3 consists of European studies with epidemiologic data on common eye disorders; a detailed description on the studies included in the consortium has been published elsewhere.[28] All studies with gradable macular fundus photographs (*N*=30,953 participants) aged 50 years and over contributed their data to the EYE-RISK database version 4.0. Studies were population-based cohort studies except for CRETEIL and EUGENDA which are clinic-based studies. Routine blood lipid measurements and AMD outcomes of the same visit were used for this analysis; for TwinsUK the closest visit to capturing of the retinal fundus photos was used. All studies were performed in accordance with the Declaration of Helsinki for research involving human subjects and the good epidemiological practice guideline.

Detailed lipid analyses

The population-based Rotterdam Study (RS) I provided data on lipid sub fractions which were determined at visit 4. Descriptive statistics of this cohort are shown in **Supplementary Table 1** (available at External link http://www.aaojournal.org).

Clinical examination:

AMD features were graded per eye on fundus photographs by experienced graders or clinicians; the most severe AMD grade classified the AMD status of the person. When needed, photographs were regraded by expert graders from Moorfields Eye Hospital and the Rotterdam Study to harmonize the outcome. AMD status was determined for all included studies using the Rotterdam Classification as previously described.[29] In brief, grade 0 or 1 are considered no AMD; grade 2 and 3 with soft indistinct drusen, reticular drusen or distinct drusen with pigmentary changes as early AMD, and grade 4 with Geographic Atrophy or Choroidal Neovascularization as late AMD. The area of the Early Treatment Diabetic Retinopathy Study grid covered by drusen was estimated in RS I visit 4 per grid circle, and calculated using previously defined harmonization criteria[30]. Medication use and lifestyle factors

including smoking habits were assessed by questionnaire; lipid measurements and other clinical determinants such as hypertension, body mass index (BMI), diabetes mellitus were examined at each individual research center (**Supplement cohort descriptions** available at External link http://www.aaojournal.org). Fasting blood draws were taken in all studies except for EUGENDA, MARS, and the Tromsø Eye Study, which drew blood samples non-fasting. Total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides were measured in plasma (POLA, PAMDI, Montrachet-3C, CRETEIL) or in serum (remaining studies) using standard operating procedures. When LDL was not measured and triglycerides were below 4.52mmol/L, a proxy was calculated using the Friedewald formula[31]: LDL-cholesterol = Total cholesterol – HDL cholesterol – (Total triglyceride ÷ 2.19); only positive values entered the analysis.

Nuclear magnetic resonance (NMR) metabolomics analysis

Lipid sub fractions were measured with the Nightingale's NMR-based biomarker platform in fasting Ethylenediaminetetraacetic acid (EDTA) plasma samples (Nightingale Ltd, Helsinki, Finland). These measurements cover multiple metabolic pathways, including lipoprotein lipids and subclasses, fatty acids, amino acids, and glycolysis-related metabolites. The NMR-based metabolic profiling has previously been described in detail[32] and has been used in multiple large-scale epidemiological and genetic studies[33-36].

Genetic analyses

The Alienor-3C study and Montrachet-3C study were genotyped with the Illumina Human 610-Quad BeadChip and imputed with the 1000 Genomes Phase I integrated variant set (March2012) using Shapeit v2.r727 (https://mathgen.stats.ox.ac.uk/genetics_software/shapeit/shapeit.html) for pre-phasing and Impute2 v2.3 (https://mathgen.stats.ox.ac.uk/impute/impute_v2.html) for imputation. Rotterdam Study I, II and III were genotyped using the Illumina 550K, 550k due/610K Illumina arrays. The genotypes were imputed with the 1000 Genomes (Phase 1 version 3) reference panel using the Markov chain Haplotyping (MaCH)/minimac software[37-39]. The EUGENDA study was genotyped with a custom-designed Illumina HumanCoreExome array within the International AMD Genetics Consortium (IAMDGC). Details regarding the design of this array, annotation, imputation and quality control of the genotypic data have

been previously described[19]. All cohorts applied similar quality control procedures to genotype data prior to analysis, imputation quality was r^2>0.3.

A total AMD genetic risk score was calculated using 33 out of the 52 known AMD risk variants[19] available in the EYE-RISK database version 4.0, see also subscript **Supplementary Table 19** (available at External link http://www.aaojournal.org). Genetic allele dosage was annotated as 0 for non-carriers, 1 for heterozygotes, and 2 for homozygotes. The genetic risk score was composed by calculating the sum of the betas of independent risk variants. The score was standardized and added as a covariate in a linear regression analysis with AMD as the dependent variable. The linear regression was corrected for age, sex, lipid lowering drugs and study site. The effect of individual lipid-related single nucleotide polymorphisms (SNP) on each lipid level or lipid sub fraction was assessed in a mixed-effects regression correcting for age, sex, lipid lowering drug usage, plasma or serum, fasting state and using study site as a random effect term. The p-value threshold for these analyses were 0.05/60 = 0.00083 ($8.3x10^{-4}$) after Bonferroni correction.

Statistical analysis

The outcome variable was presence of early or late AMD versus no AMD. Differences in baseline characteristics were evaluated with a Wald test using a logistic regression analysis, adjusting for age, gender, and study site. Analyses were conducted on complete data. Odds ratios (OR) for the routine blood lipid measurements were calculated using random-intercepts mixed-effects logistic regression models, including study site as a random effect term to allow for variability between study sites. The study site specific fixed effects estimates were transformed to their marginal counterparts as described by Heagerty and Zeger[40].

Association of HDL-cholesterol with AMD characteristics (presence of various drusen sizes, hyper- or hypopigmentation) were calculated in a univariate logistic regression analysis for the worse eye, defined as the eye with the most severe lesions of each AMD characteristic, correcting for age, sex, lipid lowering drugs usage and study site. The linear regression for HDL-cholesterol and drusen area was calculated in the Rotterdam Study I visit 4 only.

For the analysis on lipid sub fractions, all sub fractions were +1 log transformed and scaled to make comparable measurements. Association magnitudes were reported in units of standard deviation (SD) or odds ratio (OR) change per 1-SD increase in each metabolite, as previously suggested by others[34, 35]. To account for the correlation between lipid sub fractions, the eigenvalues were calculated as proposed by

Li and Ji[41] on the SNPSpD online interface[42]. Bonferroni correction was applied to correct for multiple testing using the eigenvalues to calculate the p-value threshold (p-value=0.001087). To test for differences between AMD stage and the mean of the lipid sub fractions, a Welch test was performed on the total of all age categories. The Welch test was chosen since homogeneity of variance was violated between the AMD severity classes. The post hoc Games-Howell test was used to investigate differences between the mean of the No AMD and Late AMD groups.

Mixed-effects logistic regression models were performed with R package lme4[43] and mixed-effects regression models with nmle[44] (R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/); Welch-test and genetic risk scores with SPSS (IBM Corp. Released 2012 IBM SPSS Statistics for Windows, Version 24.0 Amonk, NY: IBM Corp). Graphical outputs were constructed with GraphPad Prism 7 (GraphPad Prism version 7.00 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com").

RESULTS

We identified a total of 4,730 individuals with early AMD; 2,441 with late AMD; and 23,782 non-affected persons. The baseline characteristics of these participants are summarized in **Table 2**. AMD cases and controls differed in age, sex, BMI, lipid lowering drug use, and smoking, in accordance with the known AMD risk profile.

Routine blood lipid measurements and AMD in the E3 consortium

Next, we examined lipid levels in the entire study population. Mean levels of all lipids were within physiologic limits: mean total cholesterol ranged between studies from 5.1 mmol/L to 5.8 mmol/L; mean HDL-cholesterol from 1.4 mmol/L to 1.9 mmol/L. Mean LDL-cholesterol ranged from 3.0 mmol/L to 3.8 mmol/L; and mean triglycerides from 1.2 mmol/L to 1.7mmol/L. A fifth of the study population had collected only non-fasting blood samples; in these, the mean levels were similar but on the higher range of total cholesterol and triglycerides; 5.7 mmol/L for total cholesterol and 1.7mmol/L for triglycerides. Mean HDL-cholesterol was on the lower range with 1.5mmol/L and LDL-cholesterol was very comparable with 3.5 mmol/L.

Table 3 shows the association between lipid levels and AMD adjusted for age, sex, lipid lowering drug usage, body mass index, smoking, plasma or serum, fasting state and study site in the E3 consortium. Analyses for late AMD were also corrected for diabetes. Total cholesterol was not associated with any of the AMD outcomes. Higher HDL-cholesterol was associated with an increased risk of any AMD, and risk estimates were slightly higher for early AMD (OR 1.34 per 1 mmol/L increase), than for late AMD (OR 1.12 per 1 mmol/L increase), but had overlapping confidence intervals. LDL-cholesterol and triglycerides were inversely associated with early (OR 0.96 and OR 0.88 per 1 mmol/L increase, respectively) and any AMD (OR 0.98 and OR 0.94 per 1 mmol/L increase, respectively), but effect sizes were smaller than for HDL-cholesterol. Sensitivity analysis on fasting and non-fasting sampling methods and sex showed no interaction or it showed interaction but with similar point estimates of the odds ratios which made sampling effect or confounding unlikely. However, a sensitivity analysis on plasma or serum sampling methods did show a change in direction of effect of triglycerides measured in plasma, although not statistically significant (**Supplementary Table 4-10**, available at External link http://www.aaojournal.org). To investigate whether observed associations were the result of preferential survival of elderly without cardiovascular disease, we repeated the analyses in various age strata (**Supplementary Table 11-13**, available at External link http://www.aaojournal.org). Even in those aged 65 years or younger with AMD, HDL-cholesterol was significantly associated with increased risk of AMD (OR 1.19 per 1 mmol/L increase, p-value 0.02). LDL-cholesterol was inversely associated with AMD (OR 0.93 per 1 mmol/L, p-value 0.02), associations with triglycerides became insignificant.

Routine blood lipids measurements and early AMD phenotype

As the association between HDL-cholesterol and AMD was most pronounced in those with early AMD, we performed more detailed analyses using the various early AMD features as outcomes. Effects of HDL-cholesterol and triglycerides became larger with increasing drusen size, **Figure 1**. Likewise, higher HDL levels were associated with greater drusen area (beta 0.014; P-value=0.001); higher triglyceride levels were associated with lower drusen area. Correcting for smoking did not change these results (data not shown). Lipids were not statistically significantly associated with pigmentary changes, and total cholesterol and LDL-cholesterol were not associated with any early AMD characteristic (**Supplementary table 15 and 16**, available at External link http://www.aaojournal.org).

Lipid sub fractions in the Rotterdam Study

To explore whether the association between HDL-cholesterol, triglycerides, and AMD was driven by specific lipid sub fractions, we examined lipid sub fractions with NMR in the Rotterdam Study-I **Figure 2** (**Supplementary table 14**, available at External link http://www.aaojournal.org). The concentration of extra-large HDL particles (XL-HDL-P) was most significantly associated with any AMD, particularly the sub fractions of phospholipids and total lipids within extra-large HDL particles. These sub fractions are highly correlated (Pearson correlation >0.97). Next, total cholesterol and free cholesterol in the small VLDL were significantly associated, as well as the ratio ApoB:ApoA1, with a Pearson correlation ranging between 0.93 and 0.87. No other metabolites were significantly associated with AMD. Correcting for smoking did not change these significant results (data not shown). The ApoB-ApoA1 ratio is a surrogate for the LDL/HDL ratio with a small ratio suggesting a high level of HDL compared to LDL lipoproteins.
These associations show a dose dependent relation with AMD stages from the Rotterdam Classification **Figure 3**. To test if the mean of the lipid sub fractions per AMD stage differed statistically, we performed a Welch test, which was significant for each of the six sub fractions. The sub fractions related to HDL also showed a statistically significant difference in the Games-Howell post hoc test comparing the mean of those with no AMD with late AMD; p-value=0.01 for the concentration of extra-large HDL, p-value=0.02 for phospholipids in extra-large HDL and the p-value=0.01 for total lipids in extra-large HDL. The Games-Howell post-hoc test was not significant in the other three sub fractions, likely due to the small group size and variance in late AMD. (**Supplementary Figure 4** shows dose dependency per age category available at External link http://www.aaojournal.org.)

We also performed the analyses stratified for lipid-lowering drug use, which was reported by less cases (17.0%) than controls (24.2%) in the Rotterdam Study (p=0.02). Significance was found only in those not taking lipid-lowering drugs, and point estimates were highly similar to the overall group. (**Supplementary Table 17- 18**, available at External link http://www.aaojournal.org).

Lipid genes, lipid sub fractions and AMD

As genetic variants are an important cause of AMD, we investigated the relation between genes, lipids, and AMD. First, we investigated whether a genetic risk score with 33 SNPs covering all major AMD genes influenced lipid levels in the E3 consortium, and found with increasing genetic risk also an increase of HDL-cholesterol (p=0.03) (**Supplementary Table 19** available at External link http://www.aaojournal.org). Subsequently, we focused on the individual AMD lipid genes. In E3, the

CETP variant rs17231506 was positively associated with HDL-cholesterol levels and negatively associated with LDL-cholesterol, while both LIPC variants rs2043085 and rs2070895 were inversely associated with HDL-cholesterol. In addition, the APOE variant rs429358 was associated with decreased levels of total cholesterol, triglycerides, and LDL-cholesterol, but with increased levels of HDL-cholesterol. APOE variant rs73036519 had no significant effect on the routine lipid measurements or on the lipid sub fractions. ABCA1 variant rs2740488 only influenced total cholesterol (**Table 20A**). When restricting the analysis to lipid sub fractions in the Rotterdam Study (**Table 20B**), we found similar results for the CETP variant and the LIPC variants with all extra-large HDL sub fractions.

DISCUSSION

Routine blood lipid measurements

Based on pooled data of 30,953 participants from Western Europe, we have shown that high circulating HDL-cholesterol levels and low triglyceride levels are significantly associated with AMD. The magnitude of the effect was higher for early than for late AMD, and associations were related to drusen size and area. By focusing on lipid sub fractions, we revealed that extra-large HDL particles, small VLDL particles, and the ApoB-ApoA1 ratio, a surrogate for the LDL/HDL ratio, were dose-dependent drivers of this association. AMD risk variants in lipid genes did not provide a clear explanation, as in particular the variants in *LIPC* which increase the risk of AMD, decreased HDL-cholesterol in the systemic circulation.

Our results should be interpreted in light of the strengths and limitations of the study. The combined efforts of two European consortia enabled us to create a very large database providing the statistical power to resolve conflicting findings from previous studies. The detailed NMR lipid analysis in a subset created the opportunity to find the metabolic profile behind the lipid associations. A weakness of the consortium was the use of different protocols for blood sampling, definition of confounders, and AMD phenotyping. We addressed this issue by performing a stratified analysis on sampling methods, and found that only the associations for triglycerides changed direction of effect for plasma, albeit non-significantly. We harmonized all confounders as well as the criteria for early and late AMD, and corrected for study site in the mixed-effect models.

Many previous studies did not find a statistically significant association between lipids and AMD, but studies with the larger sample sizes often found a positive association with HDL-cholesterol and an

inverse association with triglycerides[13]. The current pooled study showed that the levels of these lipids were within physiological range in both cases and controls, and that absolute differences were small in mmol/L. However, our data suggest that an increase of HDL from the 25th percentile to the 75th percentile coincides with an AMD risk increase of about 20%. Selective survival does not appear to explain our findings, as the association was already present in the youngest age group (<= 65 yrs). The exact clinical interpretation remains to be defined. Nevertheless, the findings contribute to the understanding of AMD pathogenesis.

Animal research has provided some key insights in retinal lipid metabolism. Studies in rodents showed that most lipids in the retina are synthesized locally and up to a quarter is derived from the systemic circulation[45.] Another study in mice showed that a high fat diet increases cholesterol in the retina, but not as much as in the circulation. These results suggest that transport from the systemic circulation to the retina does take place, albeit modestly. Although LDL delivers cholesterol most efficiently from the systemic circulation to the retina, HDL-cholesterol, with ApoA-I as its major lipid component[46], does this as well via scavenger receptors[26, 47, 48]. The RPE processes the internalized lipids and subsequently secretes them again on the apical side via ABCA1 transporters into the inter-photoreceptor matrix. Thereafter, lecithin-cholesterol acyltransferase (LCAT), located at the surface of nascent HDL[49], converts free cholesterol into esterified cholesterol,[50] which are present in nascent HDL. In this way LCAT transforms nascent HDL into larger, mature HDL, while LIPC hydrolyzes phospholipids in the HDL lipoprotein[23, 51]. As suggested by Tserentsoodol *et al*[26], due to the absence of LDL in the retina, it is possible that CETP has a role in transferring esterified cholesterol between lipoproteins or photoreceptor membranes. In the inter-photoreceptor matrix, HDL functions as a transport vehicle between the RPE and the photoreceptors supporting the high synthesis and degradation of the lipid-rich photoreceptor disks[26]. The RPE maintains the lipid balance by transporting lipoproteins back to Bruchs membrane[52]. The lipid contents of these lipoproteins resemble that of LDL lipoproteins rather than HDL as it has a high abundance of esterified cholesterol, but both ApoA and ApoB[53]. It has been proposed that this large amount of esterified cholesterol acts as a barrier for lipid transport through an aging retina, thereby facilitating the formation of deposits[54]. Another mechanism proposed to form deposits is through the impairment of ABCA1 transporter of macrophages, which impairs the efflux of free cholesterol out of the macrophage. This results in senescent macrophages with high levels of cholesterol in the retina of mice[55].

Interestingly, lipoproteins appear to be closely related to the complement system, the major pathway in AMD pathogenesis. Proteomic studies have shown that HDL-lipoproteins can contain essential complement components, such as C1, C2, C3, C5 and Factor B[56-58]. One study showed that CFH and lipoproteins have competitive binding in the sub-RPE extracellular matrix, when CFH is low lipoproteins can accumulate sub-RPE[59]. By contrast, HDL can also carry complement regulators such as FH1, CFHR4 and CFHR5[60, 61]. ApoA-I attached to HDL can bind clusterin, a complement lysis inhibitor that stops the complement cascade just before the C5b-9 complex is inserted into the target[62]. These findings suggest that HDL is involved in pro-inflammatory as well as complement inhibitory tasks. Higher HDL levels may cause imbalance of the physiological homeostasis[8, 63]. Taken together, this plethora of biological leads supports the contention that HDL may play a role in the initiation of AMD. More comprehensive research into lipid metabolism in the retina is warranted.

In our study, we found elevated levels of HDL-cholesterol in the circulation and decreased levels of triglycerides in persons with AMD. In more detailed analysis, we observed a higher concentration of extra-large HDL particles with a higher total lipid and phospholipid content, which are under genetic control of CETP and LIPC. The high phospholipid content of extra-large HDL is very likely related to the larger particle size, since phospholipids compose the outer shell of the lipoprotein. CETP may exert its effect on AMD partly through systemic HDL, in line with previous Mendelian randomization studies[24, 66]. The opposing effects which we found for LIPC are less easily explained, but have been observed by others[23, 24]. This finding suggests that systemic HDL may be a biomarker rather than directly causally related to AMD. In a larger study, Kettunen *et al*[33] found more genetic effects on lipid sub fractions; variants in *CETP* and *APOE* also had a decreasing effect on the small VLDL sub fractions, while a variant in *ABCA1* increased extra-large HDL. Our smaller sample size hampered us to replicate these findings.

Where do these lipid associations fit in the chronology of AMD development? The more pronounced risk for early AMD and increasing odds ratios of HDL-cholesterol for the larger size drusen suggests that lipids play an important role at the early phase of disease. Hypothetically, intervention at this phase would be most promising in preventing blindness. We did not find statistical significance for any lipid sub fraction in only those using lipid-lowering drugs, possibly because there is no effect, but probably due to the lower power in this subgroup. Evidence from other studies indicate that statins increase HDL levels slightly[67] but reduce extra-large HDL[68], and that HDL protein composition may change as well[69]. Most epidemiologic studies do not find any effect of lipid lowering drugs on AMD[8, 70-72]; however, one study observed a slower progression of AMD in persons with a certain complement factor H risk

variant[74]. Large randomized controlled trials with long term follow-up are needed to clarify the relation between lipid lowering drugs and AMD.

In conclusion, this study showed that HDL-cholesterol and triglycerides levels are particularly associated with early AMD, mostly through the association with drusen. Extra-large HDL sub fractions seem to be drivers of this association. Whether systemic lipids directly influence lipid metabolism in the retina or whether these lipids mirror pathology in the retina is a question that remains to be answered.

FIGURE LEGENDS

Figure 1. Association of HDL-cholesterol and triglycerides with age-related macular degeneration characteristics

Figure 2. The association of metabolic variables and AMD.
Each bar represents the association with AMD, the size of the bar is the odds ratio, coloring refers to effect direction and significance. Dots indicate Bonferroni statistically significant metabolic variables corrected for age, sex and lipid lowering drugs. Labels describe the properties measured in each lipid sub fraction (*P*, concentration of particles; L, total lipids; PL, phospholipids; C, total cholesterol; CE, cholesterol esters; FC, free cholesterol; TG, triglycerides). List of abbreviations is in the annex.

Figure 3. Stage dependent relationship of the six associated lipid sub fractions with AMD.
Error bars indicate 95% confidence intervals of the mean.

Précis: (max 35 words)

HDL-cholesterol is positively associated with AMD and triglycerides negatively. This is most prominently seen in drusen as early AMD features. This association seems to be driven by larger HDL sub fractions and HDL related genetics.

REFERENCES

1. Flaxman SR, Bourne RRA, Resnikoff S, et al. Global causes of blindness and distance vision impairment 1990-2020: a systematic review and meta-analysis. Lancet Glob Health 2017;5(12):e1221-e34.
2. van Leeuwen R, Klaver CC, Vingerling JR, et al. The risk and natural course of age-related maculopathy: follow-up at 6 1/2 years in the Rotterdam study. Arch Ophthalmol 2003;121(4):519-26.
3. Joachim N, Mitchell P, Burlutsky G, et al. The Incidence and Progression of Age-Related Macular Degeneration over 15 Years: The Blue Mountains Eye Study. Ophthalmology 2015;122(12):2482-9.
4. Machalinska A, Kawa MP, Marlicz W, Machalinski B. Complement system activation and endothelial dysfunction in patients with age-related macular degeneration (AMD): possible relationship between AMD and atherosclerosis. Acta Ophthalmol 2012;90(8):695-703.
5. Mullins RF, Russell SR, Anderson DH, Hageman GS. Drusen associated with aging and age-related macular degeneration contain proteins common to extracellular deposits associated with atherosclerosis, elastosis, amyloidosis, and dense deposit disease. FASEB J 2000;14(7):835-46.
6. Haimovici R, Gantz DL, Rumelt S, et al. The lipid composition of drusen, Bruch's membrane, and sclera by hot stage polarizing light microscopy. Invest Ophthalmol Vis Sci 2001;42(7):1592-9.
7. Wang L, Clark ME, Crossman DK, et al. Abundant lipid and protein components of drusen. PLoS One 2010;5(4):e10329.
8. Cougnard-Gregoire A, Delyfer MN, Korobelnik JF, et al. Elevated high-density lipoprotein cholesterol and age-related macular degeneration: the Alienor study. PLoS One 2014;9(3):e90973.
9. Klein R, Myers CE, Buitendijk GH, et al. Lipids, lipid genes, and incident age-related macular degeneration: the three continent age-related macular degeneration consortium. Am J Ophthalmol 2014;158(3):513-24 e3.
10. Blumenkranz MS, Russell SR, Robey MG, et al. Risk factors in age-related maculopathy complicated by choroidal neovascularization. Ophthalmology 1986;93(5):552-8.
11. Paun CC, Ersoy L, Schick T, et al. Genetic Variants and Systemic Complement Activation Levels Are Associated With Serum Lipoprotein Levels in Age-Related Macular Degeneration. Invest Ophthalmol Vis Sci 2015;56(13):7766-73.
12. Cheung CMG, Gan A, Fan Q, et al. Plasma lipoprotein subfraction concentrations are associated with lipid metabolism and age-related macular degeneration. J Lipid Res 2017;58(9):1785-96.
13. Kersten E, Paun CC, Schellevis RL, et al. Systemic and ocular fluid compounds as potential biomarkers in age-related macular degeneration. Surv Ophthalmol 2018;63(1):9-39.
14. Yip JLY, Khawaja AP, Chan MPY, et al. Cross Sectional and Longitudinal Associations between Cardiovascular Risk Factors and Age Related Macular Degeneration in the EPIC-Norfolk Eye Study. Plos One 2015;10(7).
15. Aoki A, Tan X, Yamagishi R, et al. Risk Factors for Age-Related Macular Degeneration in an Elderly Japanese Population: The Hatoyama Study. Invest Ophthalmol Vis Sci 2015;56(4):2580-5.
16. van Leeuwen R, Klaver CC, Vingerling JR, et al. Cholesterol and age-related macular degeneration: is there a link? Am J Ophthalmol 2004;137(4):750-2.
17. Yang K, Wang FH, Liang YB, et al. Associations between Cardiovascular Risk Factors and Early Age-Related Macular Degeneration in a Rural Chinese Adult Population. Retina-the Journal of Retinal and Vitreous Diseases 2014;34(8):1539-53.
18. Semba RD, Cotch MF, Gudnason V, et al. Serum carboxymethyllysine, an advanced glycation end product, and age-related macular degeneration: the Age, Gene/Environment Susceptibility-Reykjavik Study. JAMA Ophthalmol 2014;132(4):464-70.
19. Fritsche LG, Igl W, Bailey JN, et al. A large genome-wide association study of age-related macular degeneration highlights contributions of rare and common variants. Nat Genet 2016;48(2):134-43.

20. Wang YF, Han Y, Zhang R, et al. CETP/LPL/LIPC gene polymorphisms and susceptibility to age-related macular degeneration. Sci Rep 2015;5:15711.
21. Wang D, Zhou J, Hou XM, et al. CETP Gene may be Associated with Advanced Age-Related Macular Degeneration in the Chinese Population. Ophthalmic Genetics 2015;36(4):303-8.
22. Yu Y, Reynolds R, Fagerness J, et al. Association of variants in the LIPC and ABCA1 genes with intermediate and large drusen and advanced age-related macular degeneration. Invest Ophthalmol Vis Sci 2011;52(7):4663-70.
23. Neale BM, Fagerness J, Reynolds R, et al. Genome-wide association study of advanced age-related macular degeneration identifies a role of the hepatic lipase gene (LIPC). Proc Natl Acad Sci U S A 2010;107(16):7395-400.
24. Burgess S, Davey Smith G. Mendelian Randomization Implicates High-Density Lipoprotein Cholesterol-Associated Mechanisms in Etiology of Age-Related Macular Degeneration. Ophthalmology 2017;124(8):1165-74.
25. Cheng CY, Yamashiro K, Chen LJ, et al. New loci and coding variants confer risk for age-related macular degeneration in East Asians. Nat Commun 2015;6:6063.
26. Tserentsoodol N, Gordiyenko NV, Pascual I, et al. Intraretinal lipid transport is dependent on high density lipoprotein-like particles and class B scavenger receptors. Mol Vis 2006;12:1319-33.
27. Hasham SN, Pillarisetti S. Vascular lipases, inflammation and atherosclerosis. Clin Chim Acta 2006;372(1-2):179-83.
28. Delcourt C, Korobelnik JF, Buitendijk GH, et al. Ophthalmic epidemiology in Europe: the "European Eye Epidemiology" (E3) consortium. Eur J Epidemiol 2016;31(2):197-210.
29. Klaver CC, Assink JJ, van Leeuwen R, et al. Incidence and progression rates of age-related maculopathy: the Rotterdam Study. Invest Ophthalmol Vis Sci 2001;42(10):2237-41.
30. Klein R, Meuer SM, Myers CE, et al. Harmonizing the classification of age-related macular degeneration in the three-continent AMD consortium. Ophthalmic Epidemiol 2014;21(1):14-23.
31. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18(6):499-502.
32. Soininen P, Kangas AJ, Wurtz P, et al. High-throughput serum NMR metabonomics for cost-effective holistic studies on systemic metabolism. Analyst 2009;134(9):1781-5.
33. Kettunen J, Demirkan A, Wurtz P, et al. Genome-wide study for circulating metabolites identifies 62 loci and reveals novel systemic effects of LPA. Nat Commun 2016;7:11122.
34. Wurtz P, Havulinna AS, Soininen P, et al. Metabolite profiling and cardiovascular event risk: a prospective study of 3 population-based cohorts. Circulation 2015;131(9):774-85.
35. Wurtz P, Makinen VP, Soininen P, et al. Metabolic signatures of insulin resistance in 7,098 young adults. Diabetes 2012;61(6):1372-80.
36. Kujala UM, Makinen VP, Heinonen I, et al. Long-term leisure-time physical activity and serum metabolome. Circulation 2013;127(3):340-8.
37. Li Y, Willer CJ, Ding J, et al. MaCH: using sequence and genotype data to estimate haplotypes and unobserved genotypes. Genet Epidemiol 2010;34(8):816-34.
38. Howie B, Fuchsberger C, Stephens M, et al. Fast and accurate genotype imputation in genome-wide association studies through pre-phasing. Nat Genet 2012;44(8):955-9.
39. Li Y, Willer C, Sanna S, Abecasis G. Genotype imputation. Annu Rev Genomics Hum Genet 2009;10:387-406.
40. Heagerty PJ, Zeger SL. Marginalized multilevel models and likelihood inference. Statistical Science 2000;15(1):1-19.
41. Li J, Ji L. Adjusting multiple testing in multilocus analyses using the eigenvalues of a correlation matrix. Heredity (Edinb) 2005;95(3):221-7.

42. Nyholt DR. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. Am J Hum Genet 2004;74(4):765-9.
43. Bates D, Machler M, Bolker BM, Walker SC. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software 2015;67(1):1-48.
44. Pinheiro JDBDSSDaRCt. nlme: Linear and Nonlinear Mixed Effects Models. 2013.
45. Lin JB, Mast N, Bederman IR, et al. Cholesterol in mouse retina originates primarily from in situ de novo biosynthesis. Journal of Lipid Research 2016;57(2):258-64.
46. Shao B, Heinecke JW. Quantifying HDL proteins by mass spectrometry: how many proteins are there and what are their functions? Expert Rev Proteomics 2018;15(1):31-40.
47. Tserentsoodol N, Sztein J, Campos M, et al. Uptake of cholesterol by the retina occurs primarily via a low density lipoprotein receptor-mediated process. Mol Vis 2006;12:1306-18.
48. Duncan KG, Hosseini K, Bailey KR, et al. Expression of reverse cholesterol transport proteins ATP-binding cassette A1 (ABCA1) and scavenger receptor BI (SR-BI) in the retina and retinal pigment epithelium. Br J Ophthalmol 2009;93(8):1116-20.
49. Chen CH, Albers JJ. Distribution of lecithin-cholesterol acyltransferase (LCAT) in human plasma lipoprotein fractions. Evidence for the association of active LCAT with low density lipoproteins. Biochem Biophys Res Commun 1982;107(3):1091-6.
50. Glomset JA. The mechanism of the plasma cholesterol esterification reaction: plasma fatty acid transferase. Biochim Biophys Acta 1962;65:128-35.
51. Brunzell JD, Zambon A, Deeb SS. The effect of hepatic lipase on coronary artery disease in humans is influenced by the underlying lipoprotein phenotype. Biochim Biophys Acta 2012;1821(3):365-72.
52. Johnson LV, Forest DL, Banna CD, et al. Cell culture model that mimics drusen formation and triggers complement activation associated with age-related macular degeneration. Proc Natl Acad Sci U S A 2011;108(45):18277-82.
53. Wang L, Li CM, Rudolf M, et al. Lipoprotein particles of intraocular origin in human Bruch membrane: an unusual lipid profile. Invest Ophthalmol Vis Sci 2009;50(2):870-7.
54. Curcio CA, Johnson M, Rudolf M, Huang JD. The oil spill in ageing Bruch membrane. Br J Ophthalmol 2011;95(12):1638-45.
55. Sene A, Khan AA, Cox D, et al. Impaired cholesterol efflux in senescent macrophages promotes age-related macular degeneration. Cell Metab 2013;17(4):549-61.
56. Gordon SM, Deng J, Lu LJ, Davidson WS. Proteomic characterization of human plasma high density lipoprotein fractionated by gel filtration chromatography. J Proteome Res 2010;9(10):5239-49.
57. Rezaee F, Casetta B, Levels JH, et al. Proteomic analysis of high-density lipoprotein. Proteomics 2006;6(2):721-30.
58. Watanabe J, Charles-Schoeman C, Miao Y, et al. Proteomic profiling following immunoaffinity capture of high-density lipoprotein: association of acute-phase proteins and complement factors with proinflammatory high-density lipoprotein in rheumatoid arthritis. Arthritis Rheum 2012;64(6):1828-37.
59. Toomey CB, Kelly U, Saban DR, Bowes Rickman C. Regulation of age-related macular degeneration-like pathology by complement factor H. Proc Natl Acad Sci U S A 2015;112(23):E3040-9.
60. Skerka C, Hellwage J, Weber W, et al. The human factor H-related protein 4 (FHR-4). A novel short consensus repeat-containing protein is associated with human triglyceride-rich lipoproteins. J Biol Chem 1997;272(9):5627-34.
61. McRae JL, Duthy TG, Griggs KM, et al. Human factor H-related protein 5 has cofactor activity, inhibits C3 convertase activity, binds heparin and C-reactive protein, and associates with lipoprotein. Journal of Immunology 2005;174(10):6250-6.

62. Rosenfeld SI, Packman CH, Leddy JP. Inhibition of the lytic action of cell-bound terminal complement components by human high density lipoproteins and apoproteins. J Clin Invest 1983;71(4):795-808.
63. Wang YF, Wang MX, Zhang XQ, et al. The Association between the Lipids Levels in Blood and Risk of Age-Related Macular Degeneration. Nutrients 2016;8(10).
64. Eren E, Yilmaz N, Aydin O. High Density Lipoprotein and it's Dysfunction. Open Biochem J 2012;6:78-93.
65. G HB, Rao VS, Kakkar VV. Friend Turns Foe: Transformation of Anti-Inflammatory HDL to Proinflammatory HDL during Acute-Phase Response. Cholesterol 2011;2011:274629.
66. Fan Q, Maranville JC, Fritsche L, et al. HDL-cholesterol levels and risk of age-related macular degeneration: a multiethnic genetic study using Mendelian randomization. Int J Epidemiol 2017.
67. Neuman MP, Neuman HR, Neuman J. Significant increase of high-density lipoprotein2-cholesterol under prolonged simvastatin treatment. Atherosclerosis 1991;91 Suppl:S11-9.
68. Wurtz P, Wang Q, Soininen P, et al. Metabolomic Profiling of Statin Use and Genetic Inhibition of HMG-CoA Reductase. Journal of the American College of Cardiology 2016;67(10):1200-10.
69. Green PS, Vaisar T, Pennathur S, et al. Combined statin and niacin therapy remodels the high-density lipoprotein proteome. Circulation 2008;118(12):1259-67.
70. van Leeuwen R, Vingerling JR, Hofman A, et al. Cholesterol lowering drugs and risk of age related maculopathy: prospective cohort study with cumulative exposure measurement. BMJ 2003;326(7383):255-6.
71. Klein R, Knudtson MD, Klein BE. Statin use and the five-year incidence and progression of age-related macular degeneration. Am J Ophthalmol 2007;144(1):1-6.
72. Maguire MG, Ying GS, McCannel CA, et al. Statin use and the incidence of advanced age-related macular degeneration in the Complications of Age-related Macular Degeneration Prevention Trial. Ophthalmology 2009;116(12):2381-5.
73. Vavvas DG, Daniels AB, Kapsala ZG, et al. Regression of Some High-risk Features of Age-related Macular Degeneration (AMD) in Patients Receiving Intensive Statin Treatment. EBioMedicine 2016;5:198-203.
74. Guymer RH, Baird PN, Varsamidis M, et al. Proof of concept, randomized, placebo-controlled study of the effect of simvastatin on the course of age-related macular degeneration. PLoS One 2013;8(12):e83759.

ACKNOWLEDGEMENTS

The generation and management of GWAS genotype data for the Rotterdam Study (RS I, RS II, RS III) was executed by the Human Genotyping Facility of the Genetic Laboratory of the Department of Internal Medicine, Erasmus MC, Rotterdam, The Netherlands. The GWAS datasets are supported by the Netherlands Organisation of Scientific Research NWO Investments (nr. 175.010.2005.011, 911-03-012), the Genetic Laboratory of the Department of Internal Medicine, Erasmus MC, the Research Institute for Diseases in the Elderly (014-93-015; RIDE2), the Netherlands Genomics Initiative (NGI)/Netherlands Organisation for Scientific Research (NWO) Netherlands Consortium for Healthy Aging (NCHA), project nr. 050-060-810. We thank Pascal Arp, Mila Jhamai, Marijn Verkerk, Lizbeth Herrera and Marjolein

Peters, MSc, and Carolina Medina-Gomez, MSc, for their help in creating the GWAS database, and Karol Estrada, PhD, Yurii Aulchenko, PhD, and Carolina Medina-Gomez, MSc, for the creation and analysis of imputed data.

The Rotterdam Study is funded by Erasmus Medical Center and Erasmus University, Rotterdam, Netherlands Organization for the Health Research and Development (ZonMw), the Research Institute for Diseases in the Elderly (RIDE), the Ministry of Education, Culture and Science, the Ministry for Health, Welfare and Sports, the European Commission (DG XII), and the Municipality of Rotterdam. The authors are grateful to the study participants, the staff from the Rotterdam Study and the participating general practitioners and pharmacists.

The GWAS genotype data for the 3C-Alienor study are managed by the RID-AGE (Risk factors and molecular determinants of aging-related diseases) group of University of Lille, Institut Pasteur de Lille, and INSERM U1167 (Lille, France). We thank Benjamin Grenier-Boley, Céline Bellenguez, Jean-Charles Lambert and Philippe Amouyel for the creation and analysis of the imputed data.

TwinsUK is funded by the Wellcome Trust, Medical Research Council, European Union, the National Institute for Health Research (NIHR)-funded BioResource, Clinical Research Facility and Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust in partnership with King's College London.

Annex

Abbreviation	Name
XXL_VLDL_C	Total cholesterol in extremely large VLDL
XXL_VLDL_CE	Cholesterol esters in extremely large VLDL
XXL_VLDL_FC	Free cholesterol in extremely large VLDL
XXL_VLDL_L	Total lipids in extremely large VLDL
XXL_VLDL_P	Concentration of extremely large VLDL
XXL_VLDL_PL	Phospholipids in extremely large VLDL
XXL_VLDL_TG	Triglycerides in extremely large VLDL
XL_VLDL_C	Total cholesterol in extra-large VLDL
XL_VLDL_CE	Cholesterol esters in extra-large VLDL
XL_VLDL_FC	Free cholesterol in extra-large VLDL
XL_VLDL_L	Total lipids in extra-large VLDL

XL_VLDL_P	Concentration of extra-large VLDL
XL_VLDL_PL	Phospholipids in extra-large VLDL
XL_VLDL_TG	Triglycerides in extra-large VLDL
L_VLDL_C	Total cholesterol in large VLDL
L_VLDL_CE	Cholesterol esters in large VLDL
L_VLDL_FC	Free cholesterol in large VLDL
L_VLDL_L	Total lipids in large VLDL
L_VLDL_P	Concentration of large VLDL
L_VLDL_PL	Phospholipids in large VLDL
L_VLDL_TG	Triglycerides in large VLDL
M_VLDL_C	Total cholesterol in medium VLDL
M_VLDL_CE	Cholesterol esters in medium VLDL
M_VLDL_FC	Free cholesterol in medium VLDL
M_VLDL_L	Total lipids in medium VLDL
M_VLDL_P	Concentration of medium VLDL
M_VLDL_PL	Phospholipids in medium VLDL
M_VLDL_TG	Triglycerides in medium VLDL
S_VLDL_CE	Cholesterol esters in small VLDL
S_VLDL_L	Total lipids in small VLDL
S_VLDL_P	Concentration of small VLDL
S_VLDL_PL	Phospholipids in small VLDL
S_VLDL_TG	Triglycerides in small VLDL
XS_VLDL_C	Total cholesterol in extra-small VLDL
XS_VLDL_CE	Cholesterol esters in extra-small VLDL
XS_VLDL_FC	Free cholesterol in extra-small VLDL
XS_VLDL_L	Total lipids in extra-small VLDL
XS_VLDL_P	Concentration in extra-small VLDL
XS_VLDL_PL	Phospholipids in extra-small VLDL
XS_VLDL_TG	Triglycerides in extra-small VLDL
IDL_C	Total cholesterol in IDL
IDL_CE	Cholesterol esters in IDL
IDL_FC	Free cholesterol in IDL
IDL_L	Total lipids in IDL
IDL_P	Concentration in IDL
IDL_PL	Phospholipids in IDL
IDL_TG	Triglycerides in IDL
L_LDL_C	Total cholesterol in large LDL
L_LDL_CE	Cholesterol esters in large LDL
L_LDL_FC	Free cholesterol in large LDL
L_LDL_L	Total lipids in large LDL
L_LDL_P	Concentration in large LDL
L_LDL_PL	Phospholipids in large LDL

L_LDL_TG	Triglycerides in large LDL
M_LDL_C	Total cholesterol in medium LDL
M_LDL_CE	Cholesterol esters in medium LDL
M_LDL_FC	Free cholesterol in medium LDL
M_LDL_L	Total lipids in medium LDL
M_LDL_P	Concentration of medium LDL
M_LDL_PL	Phospholipids in medium LDL
M_LDL_TG	Triglycerides in medium LDL
S_LDL_C	Total cholesterol in small LDL
S_LDL_CE	Cholesterol esters in small LDL
S_LDL_FC	Free cholesterol in small LDL
S_LDL_L	Total lipids in small LDL
S_LDL_P	Concentration of small LDL
S_LDL_PL	Phospholipids in small LDL
S_LDL_TG	Triglycerides in small LDL
XL_HDL_C	Total cholesterol in extra-large HDL
XL_HDL_CE	Cholesterol esters in extra-large HDL
XL_HDL_FC	Free cholesterol in extra-large HDL
XL_HDL_TG	Triglycerides in extra-large HDL
L_HDL_C	Total cholesterol in large HDL
L_HDL_CE	Cholesterol esters in large HDL
L_HDL_FC	Free cholesterol in large HDL
L_HDL_L	Total lipids in large HDL
L_HDL_P	Concentration of large HDL
L_HDL_PL	Phospholipids in large HDL
L_HDL_TG	Triglycerides in large HDL
M_HDL_C	Total cholesterol in medium HDL
M_HDL_CE	Cholesterol esters in medium HDL
M_HDL_FC	Free cholesterol in medium HDL
M_HDL_L	Total lipids in medium HDL
M_HDL_P	Concentration in medium HDL
M_HDL_PL	Phospholipids in medium HDL
M_HDL_TG	Triglycerides in medium HDL
S_HDL_C	Total cholesterol in small HDL
S_HDL_CE	Cholesterol esters in small HDL
S_HDL_FC	Free cholesterol in small HDL
S_HDL_L	Total lipids in small HDL
S_HDL_P	Concentration of small HDL
S_HDL_PL	Phospholipids in small HDL
S_HDL_TG	Triglycerides in small HDL
VLDL_C	Total cholesterol in VLDL
VLDL_D	VLDL diameter
VLDL_TG	Triglycerides in VLDL

LDL_C	Total cholesterol in LDL
LDL_D	LDL diameter
LDL_TG	Triglycerides in LDL
HDL_C	Total cholesterol in HDL
HDL_D	HDL diameter
HDL_TG	Triglycerides in HDL
HDL2_C	Total cholesterol in HDL2
HDL3_C	Total cholesterol in HDL3
Serum_C	Serum total cholesterol
Serum_TG	Serum total triglycerides
TotCho	Total cholines
Remnant_C	Remnant cholesterol (non_HDL, non_LDL _cholesterol)
ApoA1	Apolipoprotein A_I
ApoB	Apolipoprotein B
EstC	Esterfied cholesterol
FreeC	Free cholesterol
FAw3	Omega 3 fatty acids
FAw3_FA	Omega 3 fatty acids to total fatty acids ratio
FAw6	Omega 6 fatty acids
FAw6_FA	Omega 6 fatty acids to total fatty acids ratio
TotFA	Total fatty acids
FALen	Fatty acid length
UnsatDeg	Estimated degree of unsaturation
CLA	Conjugated linoleic acid
LA	Linoleic acid
MUFA	Monounsaturated fatty acids
PUFA	Polyunsaturated fatty acids
SFA	Saturated fatty acids
DHA	Docosahexaenoic acid
TG_PG	Triglycerides to phosphoglycerides ratio
CLA_FA	Conjugated linoleic acid to total fatty acids ratio
DHA_FA	Docosahexaenoic acid to total fatty acids ratio
LA_FA	Linoleic acid to total fatty acids ratio
MUFA_FA	Monounsaturated fatty acids to total fatty acids ratio
PUFA_FA	Polyunsaturated fatty acids to total fatty acids ratio
SFA_FA	Saturated fatty acids to total fatty acids ratio
DAG	Diacylglycerol
DAG_TG	Diacylglycerol to triglycerides ratio
TotPG	Total phosphoglycerides
PC	Phosphatidylcholine and other cholines
SM	Sphingomyelins
AcAce	Acetoacetate
Ace	Acetate

Ala	Alanine
Alb	Albumin
bOHBut	3_hydroxybutyrate
Cit	Citrate
Crea	Creatinine
Glc	Glucose
Gln	Glutamine
Gp	Glycoprotein acetyls
His	Histidine
Ile	Isoleucine
Lac	Lactate
Leu	Leucine
Phe	Phenylalanine
Pyr	Pyruvate
Tyr	Tyrosine
Val	Valine

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Table 1. Descriptive statistics in Rotterdam Study I, visit 4.

Descriptive statistics	Controls N=1066	Cases N=464	p-value	OR	95% CI
Sex, % female	55.5% (N=592)	57.5% (N=267)	0.83	1.03	0.82 - 1.29
Age (years)	74.7 (SD 5.9)	78.1 (SD 6.6)	**<0.0001**	1.09	1.07 - 1.11
BMI	27.5 (SD 3.9)	27.0 (SD 3.9)	0.16	0.98	0.95 – 1.01
Smoking					
Former %	57.3 % (N=598)	54.3% (N=248)	0.69	0.94	0.71 – 1.25
Current %	14.5% (N=151)	15.5% (N=71)	0.26	1.24	0.85 - 1.81
Hypertension %	85.7% (N=911)	88.4% (N=410)	0.83	0.96	0.68 – 1.36
Diabetes	15.4% (N=152)	15.6% (N=68)	0.86	0.97	0.70 – 1.34
Lipid lowering drugs	24.2% (N=257)	17.0% (N=79)	**0.02**	0.72	0.54 – 0.96

Corrected for age and sex

Table 2. Baseline data and results of logistic regression analysis of the fourteen European studies.

		Controls *N*=23782	Cases *N*=7171	*p*-value	OR	95% CI
AMD						
Early AMD			*N* = 4730			
Late AMD			*N* = 2441			
Sex, % female	Early		61.7% (N=2918)	**<0.0001**	1.21	1.13 - 1.29
	Late		59.4% (N=1449)	0.74	0.98	0.88 – 1.10
	Any	57.5% (*N*=13680)	60.9% (*N*=4367)	**<0.0001**	1.15	1.09 - 1.23
Age (years)	Early		72.7 (SD 8.4)	**<0.0001**	1.06	1.06 – 1.06
	Late		76.9 (SD 8.1)	**<0.0001**	1.12	1.11 – 1.13
	Any	68.1 (SD 8.7)	74.1 (SD 8.5)	**<0.0001**	1.08	1.07 - 1.08
BMI	Early		26.6 (SD 4.2)	**0.008**	0.99	0.98 – 1.00
	Late		26.4 (SD 4.0)	**<0.0001**	1.03	1.02 – 1.05
	Any	27.0 (SD 4.3)	26.5 (SD 4.1)	0.543	1.00	0.99 – 1.05
Smoking	Early					
Former %			40.4% (N=1843)	0.77	1.01	0.94 – 1.09
Current %			8.7% (N=399)	0.14	1.10	0.97 – 1.24
	Late					
Former %			44.8% (N=917)	**<0.0001**	1.51	1.31 - 1.75
Current %			12.3% (N=253)	**<0.0001**	3.29	2.66 – 4.07
	Any					
Former %		41.3% (*N*=9530)	41.7% (*N*=2760)	**0.02**	1.09	1.01 - 1.17
Current %		12.8% (*N*=2947)	9.9% (*N*=652)	**<0.0001**	1.37	1.22 - 1.53
Hypertension %	Early		48.7% (N=2153)	0.30	1.04	0.97– 1.12
	Late		45.3% (N=977)	0.84	1.01	0.90 – 1.14
	Any	49.0% (*N*=11010)	47.6% (*N*=3130)	0.43	1.03	0.96 - 1.10
Diabetes	Early		9.7% (N=435)	0.35	0.95	0.84 – 1.06
	Late		13.2% (N=284)	**0.002**	1.33	1.11 – 1.58
	Any	10.7% (*N*=2408)	10.8% (*N*=719)	0.70	1.02	0.92 - 1.13
Lipid lowering drugs	Early		24.7% (N=1084)	**0.006**	0.89	0.83 – 0.97
	Late		22.5% (N=459)	0.86	0.99	0.85 – 1.14
	Any	24.5% (*N*=5492)	24.0% (*N*=1543)	**0.004**	0.90	0.83 - 0.97

Odds ratios are corrected for age, sex and study site. AMD = age-related macular degeneration, BMI= body mass index, OR = odds ratio

Table 3. Mixed-effects logistic regression associations of blood lipids with age-related macular degeneration

Lipid		Controls Median (25th and 75th percentile)	Cases Median (25th and 75th percentile)	OR for 1 mmol/L increase	95% CI	p-value
Total Cholesterol	Early AMD	5.60 (4.90-6.30) N=20555	5.58 (4.80-6.30) N=3907	0.98	0.95-1.01	0.24
	Late AMD	5.60 (4.90-6.30) N=20234	5.66 (4.90-6.50) N=1620	1.03	0.997-1.07	0.07
	Any AMD	5.60 (4.90-6.30) N=20555	5.60 (4.80-6.34) N=5538	1.00	0.97-1.02	0.67
HDL-cholesterol	Early AMD	1.40 (1.16-1.69) N=19931	1.50 (1.23-1.80) N=3802	1.34	1.22-1.48	**6.48×10^{-10}**
	Late AMD	1.40 (1.16-1.69) N=19662	1.47 (1.20-1.79) N=1626	1.12	1.002-1.24	0.044
	Any AMD	1.40 (1.16–1.69) N=19931	1.50 (1.22-1.80) N=5439	1.21	1.14-1.29	**1.35×10^{-9}**
LDL-cholesterol	Early AMD	3.49 (2.84-4.14) N=19590	3.37 (2.71-4.02) N=3746	0.96	0.92-0.999	0.045
	Late AMD	3.49 (2.85-4.14) N=19334	3.45 (2.79-4.14) N=1580	1.01	0.97-1.06	0.51
	Any AMD	3.49 (2.84-4.14) N=19590	3.39 (2.74-4.07) N=5337	0.98	0.95-1.01	0.13
Triglycerides	Early AMD	1.34 (1.00-1.87) N=19539	1.30 (0.97-1.80) N=3768	0.88	0.84-0.92	**2.44×10^{-7}**
	Late AMD	1.34 (1.00-1.87) N=19474	1.43 (1.01-2.01) N=1601	1.01	0.97-1.06	0.57
	Any AMD	1.34 (1.00-1.87) N=19539	1.32 (0.98-1.86) N=5374	0.94	0.91-0.97	**2.35×10^{-5}**

Odds ratio estimates and 95% confidence intervals of lipid on early, late or any AMD after adjusting for age, sex, lipid lowering drug usage, body mass index, smoking, plasma or serum, fasting state and study site. Late AMD was also corrected for diabetes.
IQR = inter quartile range. p=0.0042 is Bonferroni significant.

Table 4. Mixed-effects logistic regression for any age-related macular degeneration with interaction terms with fasting vs. non-fasting

Interaction term	OR	95% CI	p-value
Total Cholesterol *fasting/non-fasting	1.00	0.96 – 1.04	0.94
HDL-cholesterol*fasting/non-fasting	0.85	0.76 – 0.95	**0.0054**
LDL-cholesterol*fasting/non-fasting	1.01	0.96 – 1.06	0.77
Triglycerides *fasting/non-fasting	1.06	1.004- 1.13	**0.038**

Corrected for age, sex, lipid lowering drug usage, BMI, smoking, study site, plasma or serum and fasting state. Non-fasting = 1, fasting =0.

Table 5. Mixed-effects logistic regression for any age-related macular degeneration in fasting samples

Lipid	Controls Median (IQR)	Cases Median (IQR)	OR	95% CI	p-value
Total Cholesterol	5.60 (1.40) N=17122	5.50 (1.40) N=3096	0.99	0.96 – 1.01	0.28
HDL-cholesterol	1.38 (0.51) N=16498	1.50 (0.58) N=2979	1.25	1.16 – 1.34	**4.67×10^{-9}**
LDL-cholesterol	3.48 (1.28) N=16184	3.37 (1.29) N=2933	0.97	0.94 – 0.998	0.04
Triglycerides	1.32 (0.86) N=16106	1.23 (0.79) N=2942	0.93	0.89-0.96	**3.63×10^{-5}**

Corrected for age, sex, lipid lowering drug usage, BMI, smoking, study site and plasma or serum.

Table 6. Mixed-effects logistic regression for any age-related macular degeneration in non-fasting samples

Lipid	Controls Median (IQR)	Cases Median (IQR)	OR	95% CI	p-value
Total Cholesterol	5.70 (1.50) N=3433	5.70 (1.60) N=2442	1.03	0.97 – 1.08	0.34
HDL-cholesterol	1.50 (0.58) N=3433	1.49 (0.56) N=2460	1.21	1.06 – 1.40	**0.006**
LDL-cholesterol	3.50 (1.38) N=3406	3.40 (1.37) N=2404	1.01	0.95 – 1.07	0.74
Triglycerides	1.43 (0.95) N=3433	1.49 (0.97) N=2432	0.95	0.89 – 1.01	0.11

Corrected for age, sex, lipid lowering drug usage, BMI, smoking, study site and plasma or serum.

Table 7. Mixed-effects logistic regression for any age-related macular degeneration GLMM with interaction terms for plasma or serum samples

Interaction term	OR	95% CI	p-value
Total Cholesterol * plasma serum	0.97	0.90 – 1.04	0.43
HDL-cholesterol *plasma serum	1.03	0.85 – 1.25	0.77
LDL-cholesterol *plasma serum	0.98	0.89 – 1.06	0.56
Triglycerides *plasma serum	0.90	0.81- 1.01	**0.044**

Corrected for age, sex, lipid lowering drug usage, BMI, smoking, fasting state, study site and plasma or serum. Plasma serum is coded as; plasma = 0, serum =1.

Table 8. Mixed-effects logistic regression associations of **PLASMA** lipids with age-related macular degeneration

Lipid		Controls Median (IQR)	Cases Median (IQR)	OR for 1 mmol/L increase	95% CI	p-value
Total Cholesterol	Early AMD	5.68 (5.03-6.38) N=2769	5.75 (5.07-6.46) N=359	1.05	0.94-1.17	0.393
	Late AMD	5.71 (5.06-6.40) N=2539	5.66 (5.03-6.31) N=348	1.00	0.95-1.06	0.88
	Any AMD	5.68 (5.03-6.38) N=2769	5.69 (5.06-6.38) N=713	1.01	0.97-1.05	0.53
HDL-cholesterol	Early AMD	1.42 (1.17-1.68) N=2711	1.50 (1.24-1.80) N=358	1.26	0.92-1.71	0.133
	Late AMD	1.41 (1.17-1.68) N=2532	1.68 (1.33-2.07) N=347	1.09	0.91-1.29	0.35
	Any AMD	1.42 (1.17-1.68) N=2711	1.57 (1.28-1.93) N=711	1.09	0.97-1.21	0.15
LDL-cholesterol	Early AMD	3.69 (3.08-4.30) N=2687	3.62 (3.04-4.36) N=355	1.01	0.90-1.14	0.814
	Late AMD	3.71 (3.11-4.31) N=2520	3.64 (3.09-4.31) N=347	0.98	0.91-1.05	0.55
	Any AMD	3.69 (3.08-4.30) N=2687	3.63 (3.07-4.34) N=708	1.00	0.96-1.04	0.96
Triglycerides	Early AMD	1.07 (0.79-1.48) N=2546	1.03 (0.78-1.35) N=336	1.04	0.87-1.21	0.671
	Late AMD	1.07 (0.79-1.48) N=2539	1.01 (0.75-1.41) N=347	1.04	0.95-1.09	0.30
	Any AMD	1.07 (0.79-1.48) N=2546	1.02 (0.76-1.39) N=683	1.02	0.97-1.07	0.41

Odds ratio estimates and 95% confidence intervals of lipid on early, late or any AMD after adjusting for age, sex, lipid lowering drug usage, body mass index, smoking, plasma or serum, fasting state and study site. Late AMD was also corrected for diabetes.
IQR = inter quartile range. p=0.0042 is Bonferroni significant.

Table 9. Mixed-effects logistic regression associations of **SERUM** lipids with age-related macular degeneration

Lipid		Controls Median (IQR)	Cases Median (IQR)	OR for 1 mmol/L increase	95% CI	p-value
Total Cholesterol	Early AMD	5.60 (4.88-6.30) N=17786	5.53 (4.80-6.30) N=3548	0.97	0.93-1.01	0.09
	Late AMD	5.60 (4.88-6.30) N=17695	5.66 (4.80-6.50) N=1272	1.07	1.00-1.14	0.03
	Any AMD	5.60 (4.88-6.30) N=17786	5.60 (4.80-6.31) N=4825	0.99	0.95-1.02	0.43
HDL-cholesterol	Early AMD	1.40 (1.16-1.69) N=17220	1.50 (1.23-1.80) N=3444	1.36	1.23-1.50	**1.11×10^{-9}**
	Late AMD	1.40 (1.16-1.69) N=17130	1.42 (1.19-1.70) N=1279	1.19	0.99-1.42	0.06
	Any AMD	1.40 (1.16-1.69) N=17220	1.48 (1.22-1.78) N=4728	1.31	1.20-1.44	**4.12×10^{-9}**
LDL-cholesterol	Early AMD	3.45 (2.80-4.11) N=16903	3.34 (2.69-3.98) N=3391	0.95	0.91-0.99	0.014
	Late AMD	3.45 (2.80-4.11) N=16814	3.39 (2.74-4.11) N=1233	1.04	0.97-1.12	0.26
	Any AMD	3.45 (2.80-4.11) N=16903	3.35 (2.70-4.02) N=4629	0.96	0.93-1.003	0.07
Triglycerides	Early AMD	1.40 (1.02-1.91) N=16993	1.32 (1.00-1.80) N=3432	0.87	0.83-0.91	**4.49×10^{-8}**
	Late AMD	1.40 (1.02-1.92) N=16925	1.56 (1.12-2.17) N=1254	1.01	0.94-1.09	0.72
	Any AMD	1.40 (1.02-1.91) N=16993	1.40 (1.00-1.90) N=4691	0.90	0.86-0.94	**5.07×10^{-6}**

Odds ratio estimates and 95% confidence intervals of lipid on early, late or any AMD after adjusting for age, sex, lipid lowering drug usage, body mass index, smoking, plasma or serum, fasting state and study site. Late AMD was also corrected for diabetes.
IQR = inter quartile range. p=0.0042 is Bonferroni significant.

Table 10. Mixed-effects logistic regression for any age-related macular degeneration GLMM with interaction terms for gender

Interaction terms	OR	95% CI	p-value
Total Cholesterol *gender	0.96	0.92-1.01	0.11
HDL-cholesterol * gender	1.08	0.95-1.22	0.24
LDL-cholesterol * gender	0.95	0.91-1.001	0.053
Triglycerides * gender	0.96	0.91-1.02	0.19

Corrected for age, sex, lipid lowering drug usage, BMI, smoking, fasting state, study site and plasma or serum. gender is coded: 0= male, 1 = female.

Table 11. Mixed-effects logistic regression for any age-related macular degeneration for participants aged ≤ 65

Lipid	Controls Median (IQR)	Cases Late AMD Median (IQR)	OR	95% CI	p-value
Total Cholesterol	5.62 (1.36) N=8606	5.60 (1.48) N=955	0.96	0.91 – 1.01	0.09
HDL-cholesterol	1.39 (0.53) N=8310	1.48 (0.59) N=924	1.19	1.02– 1.38	**0.02**
LDL-cholesterol	3.50 (1.27) N=8188	3.40 (1.30) N=907	0.93	0.87 – 0.99	**0.02**
Triglycerides	1.34 (0.91) N=8139	1.34 (0.88) N=918	0.95	0.89- 1.02	0.15

Corrected for age, sex, lipid lowering drug usage, BMI, smoking, fasting state, study site and plasma or serum.

Table 12. Mixed-effects logistic regression for any age-related macular degeneration for participants aged > 65 & ≤ 80

Lipid	Controls Median (IQR)	Cases Late AMD Median (IQR)	OR	95% CI	p-value
Total Cholesterol	5.59 (1.44) N=10030	5.60 (1.51) N=3258	1.01	0.98– 1.04	0.60
HDL-cholesterol	1.40 (0.52) N=9775	1.50 (0.56) N=3199	1.25	1.15– 1.36	**1.62×10^{-07}**
LDL-cholesterol	3.48 (1.33) N=9587	3.40 (1.34) N=3146	1.00	0.96 – 1.03	0.80
Triglycerides	1.37 (0.90) N=9570	1.35 (0.89) N=3160	0.92	0.89- 0.96	**6.22×10^{-05}**

Corrected for age, sex, lipid lowering drug usage, BMI, smoking, fasting state, study site and plasma or serum.

Table 13. Mixed-effects logistic regression for any age-related macular degeneration for participants aged > 80

Lipid	Controls Median (IQR)	Cases Median (IQR)	OR	95% CI	p-value
Total Cholesterol	5.56 (1.45) N=1919	5.50 (1.59) N=1325	1.00	0.94 – 1.06	0.93
HDL-cholesterol	1.44 (0.56) N=1846	1.47 (0.55) N=1316	1.24	1.04 – 1.47	**0.02**
LDL-cholesterol	3.43 (1.30) N=1815	3.38 (1.34) N=1284	0.98	0.91 – 1.05	0.62
Triglycerides	1.24 (0.76) N=1830	1.30 (0.84) N=1296	0.96	0.87-1.06	0.41

Corrected for age, sex, lipid lowering drug usage, BMI, smoking, fasting state, study site and plasma or serum.

Table 14. Univariable logistic regression for AMD for each lipid sub fraction, corrected for age, sex and lipid lowering drugs, sorted on p-value

Lipid sub fraction	p-value	OR	95%CI
Concentration extra-large HDL	**4.06×10^{-4}**	1.24	1.10-1.40
Phospholipids in extra-large HDL	**4.32×10^{-4}**	1.24	1.10-1.40
Total cholesterol in small VLDL	**7.36×10^{-4}**	0.81	0.72-0.92
Ratio ApoB ApoA1	**7.65×10^{-4}**	0.82	0.730.92
Total lipids in extra-large HDL	**7.77×10^{-4}**	1.23	1.10-1.39
Free cholesterol in small VLDL	**7.80×10^{-4}**	0.81	0.72-0.92
Total cholesterol in extra-large HDL	1.40×10^{-3}	1.22	1.08-1.37
Cholesterol esters in extra-large HDL	1.50×10^{-3}	1.22	1.08-1.37
Cholesterol esters in small VLDL	1.56×10^{-3}	0.82	0.73-0.93
Total cholesterol in VLDL	1.74×10^{-3}	0.82	0.73-0.93

Bold p-values are Bonferroni significant, OR is for 1-SD. Lowest 10 p-values are shown.

Table 15. Association of **LDL** with age-related macular degeneration characteristics

AMD characteristic	Not present LDL Mean (+/-SD)	Present LDL Mean (+/-SD)	OR	95% CI	p-value
Small drusen <63um	3.25 (0.97) N=7079	3.59 (0.93) N=3295	0.99	0.93-1.05	0.78
Intermediate drusen >63um and <125um	3.55 (0.94) N=5651	3.62 (0.95) N=3254	0.95	0.90-1.00	0.07
Large drusen >125um	3.44 (0.98) N=11883	3.43 (0.99) N= 2066	0.99	0.94-1.05	0.74
Hyper pigmentation	3.59 (0.95) N=12982	3.48 (0.94) N=1578	0.95	0.88-1.01	0.09
Hypo pigmentation	3.58 (0.94) N=13027	3.52 (0.95) N=1531	0.98	0.92-1.05	0.56

Corrected for age, sex, lipid lowering drugs and study site

Table 16. Association of **total cholesterol** with age-related macular degeneration characteristics

AMD characteristic	Not present Total cholesterol Mean (+/-SD)	Present Total cholesterol Mean (+/-SD)	OR	95% CI	p-value
Small drusen <63um	5.40 (1.09) N=7890	5.73 (1.01) N=3450	0.99	0.94-1.04	0.67
Intermediate drusen >63um and <125um	5.56 (1.03) N=6408	5.72 (1.04) N=3412	0.97	0.93-1.02	0.24
Large drusen >125um	5.55 (1.08) N=12733	5.58 (1.08) N= 2205	1.02	0.98-1.07	0.32
Hyper pigmentation	5.63 (1.04) N=13950	5.61 (1.04) N=1646	0.97	0.91-1.02	0.24
Hypo pigmentation	5.63 (1.04) N=14004	5.64 (1.06) N=1590	1.00	0.95-1.06	0.98

Corrected for age, sex, lipid lowering drugs and study site

Table 17. Univariable logistic regression for each lipid sub fraction, corrected for age, sex and lipid lowering drugs, sorted on p-value only participants using lipid lowering drugs.

Variable	p-value	OR	95%CI
Pyruvate(mmol/l)	0.005835	0.66	0.49-0.8
Albumin(signal area)	0.00821	0.69	0.52-0.90
Alanine(mmol/l)	0.013313	0.70	0.52-0.92
Tyrosine(mmol/l)	0.04998	0.76	0.57-0.99
Total lipids in small HDL	0.058837	0.76	0.57-1.01
Concentration of small HDL particles	0.060846	0.76	0.57-1.01
Diacylglycerol(mmol/l)	0.126999	0.80	0.60-1.05
Lactate(mmol/l)	0.136759	0.81	0.61-1.06
Triglycerides in extra large HDL	0.139265	1.22	0.94-1.60
Total cholesterol in small HDL	0.153029	0.82	0.62-1.08

Table 18. Univariable logistic regression for each lipid sub fraction, corrected for age, sex and lipid lowering drugs, sorted on p-value only in participants not using lipid lowing drugs

Variable	p-value	OR	95%CI
Total cholesterol in small VLDL	**0.000806**	0.796	0.695-0.908
ApoB - ApoA1 Ratio	**0.000808**	0.802	0.705-0.912
Phospholipids in extra-large HDL	**0.001003**	1.246	1.093-1.422
Concentration of extra- large HDL	0.001074	1.243	1.091-1.416
Total lipids in extra small VLDL	0.00109	0.801	0.700-0.914
Free cholesterol in small VLDL	0.001098	0.802	0.701-0.914
Total cholesterol in extra small VLDL	0.001122	0.803	0.703-0.915
Concentration of extra small VLDL	0.001232	0.803	0.702-0.916
Cholesterol esters in small VLDL	0.001387	0.804	0.702-0.918
Total cholesterol in VLDL	0.001516	0.806	0.704-0.919

Table 19 Univariable analysis of the genetic risk score for each routine lipid measurement. .

Lipid sub fractions	Estimate per 1 SD	Std. Error	p-value
Total cholesterol	-0.019	0.015	0.188
HDL-cholesterol	0.012	0.005	**0.031**
LDL-cholesterol	-0.025	0.013	0.056
Triglycerides	-0.011	0.012	0.365

AMD_Risk_score = (CFH_rs10922109 * 0.673344553) + (CFH_rs570618 * 0.553885113) + (CFH_rs148553336 * 1.171182982) + (CFH_rs187328863 * 0.385262401) + (CFH_rs35292876 * 0.431782416) + (CFH_rs191281603 * 0.891598119) + (COL4A3_rs11884770 * 0.083381609) + (ADAMTS9_AS2_rs62247658 * 0.131028262) + (COL8A1_rs140647181 * 0.615185639) + (COL8A1_rs55975637 * 0.148420005) + (CFI_rs10033900 * 0.139761942) + (C9_rs62358361 * 0.512823626) + (C2_CFB_SKIV2L_rs181705462 * 0.444685821) + (C2_CFB_rs943080 * 0.139262067) + (KMT2E_SRPK2_rs1142 * 0.131028262) + (PILRB_PILRA_rs7803454 * 0.139761942) + (MIR6130_RORB_rs10781182 * 0.104360015) + (TGFBR1_rs1626340 * 0.127833372) + (ARHGAP21_rs12357257 * 0.113328685) + (B3GALTL_rs9564692 * 0.105360516) + (RAD51B_rs61985136 * 0.127833372) + (RAD51B_rs2842339 * 0.165514438) + (LIPC_rs2043085 * 0.139761942) + (C2_CFB_SKIV2L_rs144629244 * 1.026041596) + (LIPC_rs2070895 * 0.15082289) + (CETP_rs17231506 * 0.104360015) + (TMEM97_VTN_rs11080055 * 0.083381609) + (APOE_rs429358 * 0.400477567) + (APOE_rs73036519 * 0.094310679) + (SYN3_TIMP3_rs5754227 * 0.235722334) + (SLC16A8_rs8135665 * 0.131028262) + (ABCA1_rs2740488 * 0.116533816) + (ARMS2_HTRA1_rs3750846 * 1.075002423).

The risk score is standardized and estimates are corrected for age, sex, lipid lowering drugs, serum or plasma, fasting state and study site.

Table 20A. Mixed-effects linear regression model estimating the effect of SNPs on routine lipid measurements.

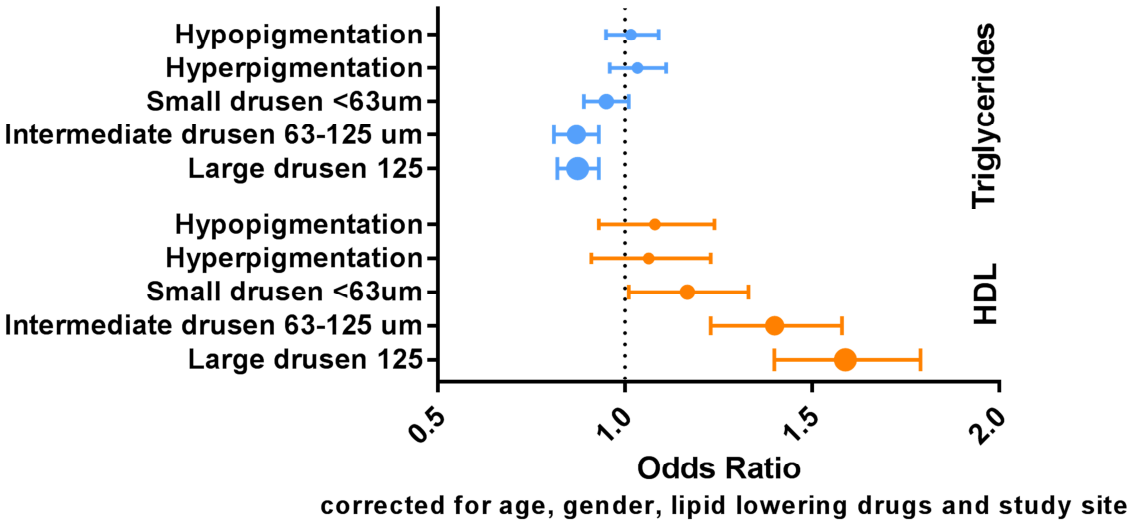
Lipid	*CETP* rs17231506 Risk allele T, reference allele C	*LIPC* rs2043085 Risk allele C, reference allele T	*LIPC* rs2070895 Risk allele G, reference allele A	*APOE* rs429358 Risk allele T, reference allele C	*APOE* rs73036519 Risk allele G, reference allele C	*ABCA1* rs2740488 Risk allele A, reference allele C
Total cholesterol	0.03 (p=0.07)	-0.019 (p=0.16)	-0.04 (p=0.008)	**-0.18 (p< 0.0001)**	0.01 (p=0.55)	**0.06 (p=0.0002)**
HDL-cholesterol	**0.08 (p< 0.0001)**	**-0.04 (p< 0.0001)**	**-0.05 (p< 0.0001)**	**0.03 (p< 0.0001)**	-0.007 (p=0.24)	**0.03 (p=0.0001)**
LDL-cholesterol	**-0.05 (p=0.0001)**	0.02 (p=0.09)	0.004 (p=0.77)	**-0.19 (p< 0.0001)**	0.01 (p=0.67)	0.04 (p=0.01)
Triglycerides	-0.025 (p=0.04)	0.001 (p=0.93)	-0.006 (p=0.66)	**-0.06 (p=0.0004)**	0.03 (p=0.03)	0.004 (p=0.78)

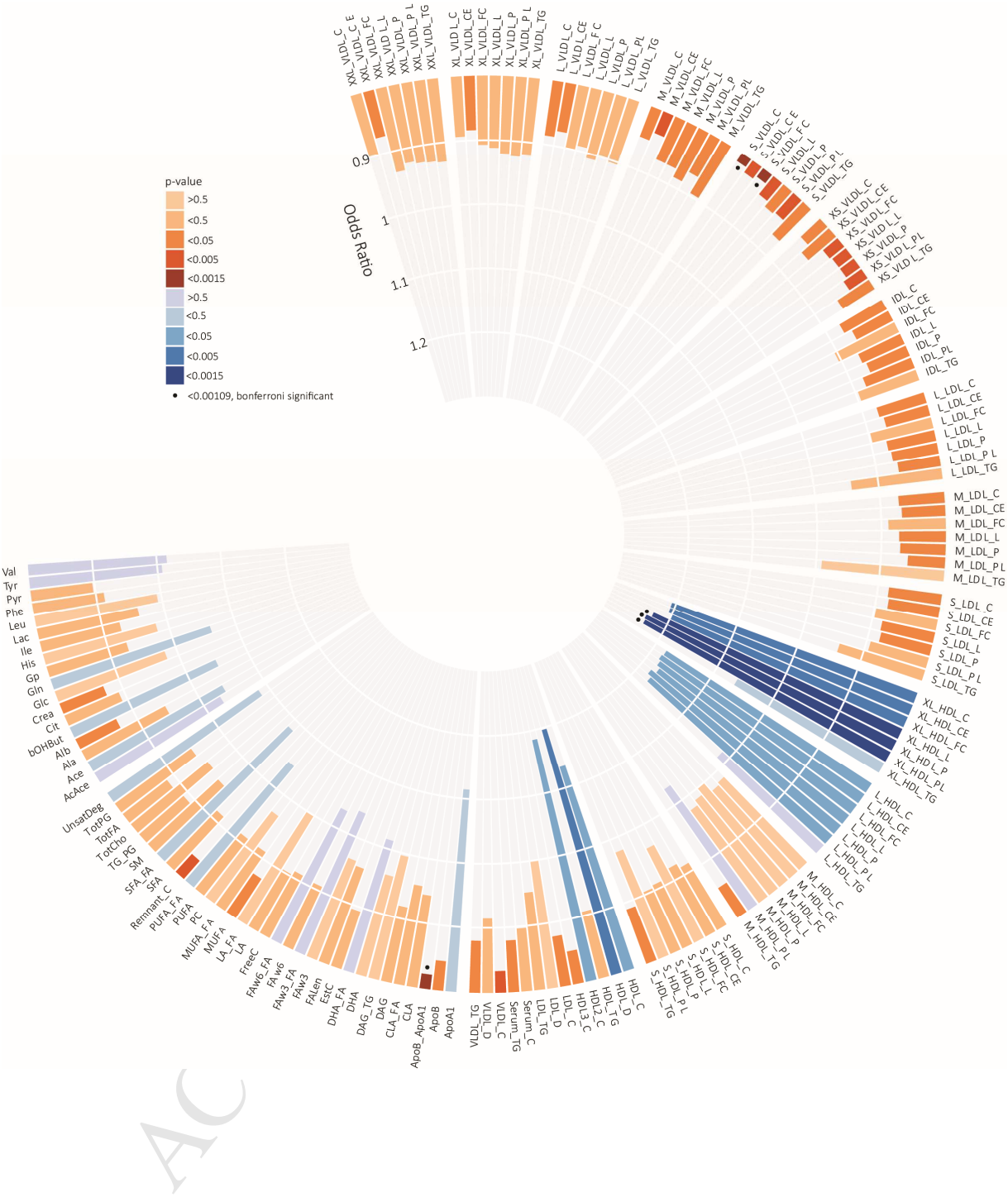
Betas are corrected for age, gender, lipid lowering drugs, plasma or serum, fasting state and study site. Betas indicate the effect of the risk allele versus the reference allele. Bonferroni: 0.05/60 = 0.00083 (8.3x10-4). Red is negative effect size, blue is positive effect size.

Table 20B. Linear regression model estimating the effect of SNPs on lipid sub fraction.

Lipid	*CETP* rs17231506 Risk allele T, reference allele C	*LIPC* rs2043085 Risk allele C, reference allele T	*LIPC* rs2070895 Risk allele G, reference allele A	*APOE* rs429358 Risk allele T, reference allele C	*APOE* rs73036519 Risk allele G, reference allele C	*ABCA1* rs2740488 Risk allele A, reference allele C
Percentage extra-large HDL	**0.15 (p= $7.68x10^{-7}$)**	**-0.14 (p= $1.00x10^{-6}$)**	**-0.13 (p= $1.55x10^{-4}$)**	-0.07 (p= 0.112)	0.04 (p=0.167)	0.05 (p=0.164)
Phospholipids in extra-large HDL	**0.15 (p= $3.51x19^{-7}$)**	**-0.13 (p= $4.0x10^{-6}$)**	**-0.13 (p= $1.04x10^{-4}$)**	-0.03 (p= 0.479)	0.04 (p=0.233)	0.03 (p=0.349)
Total cholesterol in small VLDL	-0.09 (p=0.003)	-0.09 (p=0.003)	-0.08 (p=0.02)	-0.05 (p=0.294)	-0.02 (p=0.631)	0.08 (p=0.027)
Ratio ApoB ApoA1	-0.10 (p=0.002)	0.004 (p=0.897)	-0.003 (p=0.936)	-0.09 (p=0.037)	-0.04 (p=0.250)	0.02 (p=0.521)
Total lipids in extra-large HDL	**0.15 (p=$4.06x10^{-7}$)**	**-0.14 (p=$8.59x10^{-7}$)**	**-0.12 (p=$3.13x10^{-4}$)**	-0.08 (p=0.056)	0.04 (p=0.213)	0.05 (p=0.138)
Free cholesterol in small VLDL	-0.08 (p=0.01)	-0.08 (p=0.005)	-0.09 (0.009)	0.01 (p=0.784)	-0.03 (p=0.434)	0.07 (p=0.058)

Betas are corrected for age, gender, lipid lowering drug usage, sub fractions are log+1 and standardized. Betas indicate the effect of the risk allele versus the reference allele.





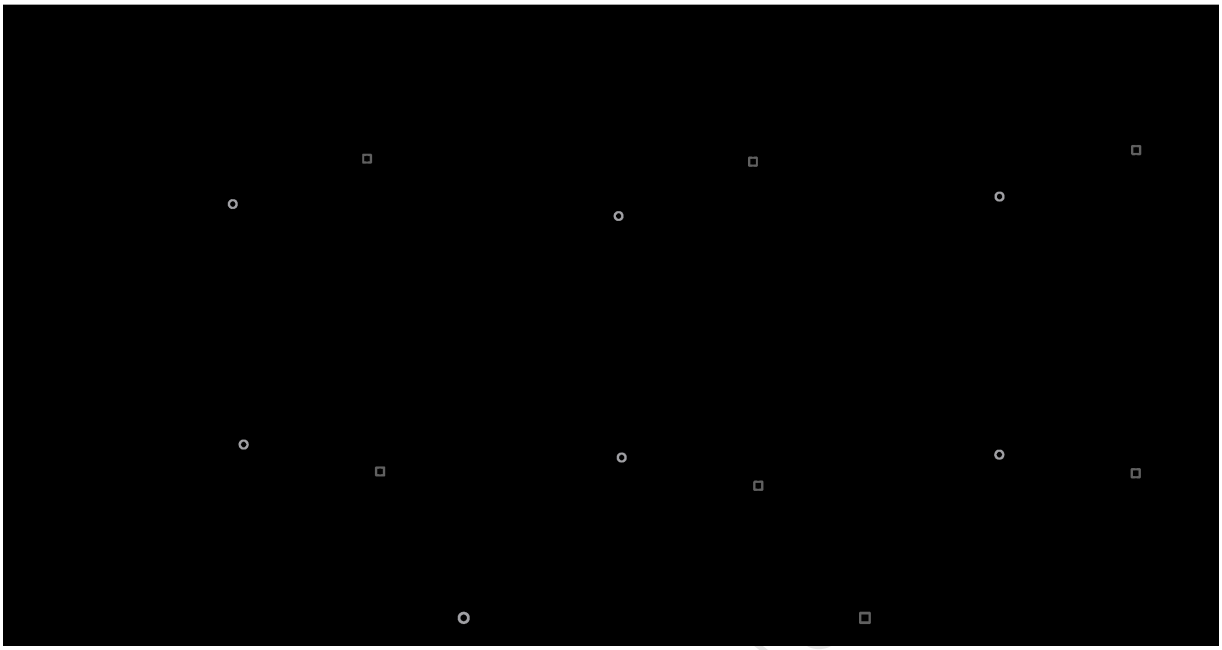
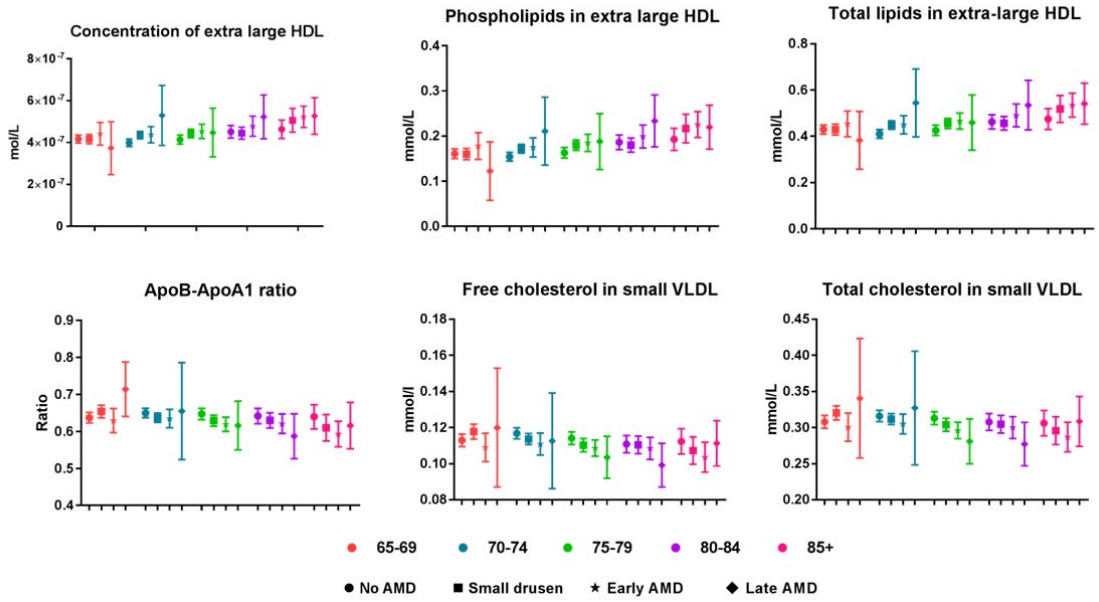


Figure 4. Dose dependent relationship of the six associated lipid sub fractions with AMD per age category. Error bars indicate 95% confidence intervals of the mean.



Précis:

HDL-cholesterol is positively associated with AMD and triglycerides negatively. This is most prominently seen in drusen as early AMD features. This association seems to be driven by larger HDL sub fractions and HDL related genetics.